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Phytochemical constituents of *Terminalia arjuna* from Chandrapur district of Maharashtra, India.

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Abstract

Plants contain many bioactive compounds which provide medicinal value to cure certain diseases. The aim of the study was to determine the biologically active phytochemical constituents of *Terminalia arjuna* from Chandrapur district of Maharashtra. Also, the quantitative estimation of total phenol, tannins and flavonoids were carried out to validate the medicinal value of the plant. The extract of leaves, bark, and fruits were prepared using three solvent i.e., distilled water, methanol and ethanol and the total extractive value was calculated. The highest extractive value found in ethanolic bark extract and lowest in methanolic leaf extract. The qualitative phytochemical analysis reveals the presence of many primary and secondary metabolites like proteins, carbohydrates, phenols, tannins, alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids, quinines, coumarins and pseudo tannins etc. The highest concentration of total phenols was found in bark and lowest in fruits. Tannin content was found comparatively higher in leaves and lower in fruits. Flavonoids were recorded highest in bark whereas it was lowest in fruits.

Keywords: *Terminalia arjuna,* extractive value, Total phenols, Tannins, Flavonoids.

1. Introduction

Phytochemistry is the science which has been used in ancient times all over the world. In eastern countries, the well established records are available from Vedas to Ayurvedas and also in western countries the records are available from the time of Theophrastus – Father of Botany [1]. The plants are having

the huge treasures of phytochemical components and factors that can be one of the best remedies for the several diseases and health problems of day to day life. These chemicals were named "secondary metabolites" by A. Kossel in 1891. A great majority of these compounds do not directly participate in growth, development and reproduction of plants but these are responsible for economically valuable products such as fragrances, drugs, dyes, flavors, insecticides etc. [2].

An area of Chandrapur district is chosen for study, lies on the east in Maharashtra State in India. It is a part of the Vidharbha region in Maharashtra. Located between 18°41' to 20° 50' North latitudes and 78°48' to 80°55' East longitudes. The total area of the district is 11443 sq. kms. which is about 3.5% of the total area of Maharashtra State. More than 35% of the total geographical area of Chandrapur district consists of dense forest [3].

Terminalia arjuna is a evergreen tall dry deciduous tree having many medicinal properties and plant parts being used since ancient times in many remedies like fracture, ulcers, leucorrhea, diabetes, anemia, cardiopathy, and cirrhosis [4]. Bark powder boiled with water, and inhaled it to cure headache and to kill worms in teeth, also used as ulcer wash. Fruit paste is used to cure wounds and sores [5, 6]. Bark ashes have been prescribed for snakebite and scorpion sting [7]. Fresh leaf juice is used for the treatment of earache and bark powder for treating heart ailments [8].

2. Materials and Method

Collection and processing of samples of matutre plant:
The samples of mature plants were collected from different sites of Chandrapur forest area. The material was identified at Botany Department, Institute of Science, Nagpur and the specimen was deposited. The samples of selected plant species of different parts such as bark, leaves, fruits were collected at their mature stage of development. Then these were carefully examined and insect damaged, fungus-infested samples were removed. Only healthy samples were washed with water and then soaked with filter paper. Then the samples were spread out and shade dried at room temperature for about 10 to 20 days up to complete drying until they broke easily by hand. The samples were powdered using mortar and pestle and electrical grinder.

Method of extraction:
Bark, leaf and fruit powder used to extract with different solvents like distilled water, ethanol and methanol with the help of Soxhlet extraction apparatus. A known mass of sample was used against a known volume of solvent. After complete extraction, the extractive value was calculated for each extract as per standard procedure [9].

Extractive value (%) = (Weight of dried extract /Weight of plant material) X 100
The extracts were stored in refrigerator for further use.

Phytochemical Analysis:
Phytochemical screening was performed to identify phytochemicals in the Aqueous, Methanolic and Ethanolic extracts of plant parts used in the study in this present work by the standard methods [10, 11, 12].

Estimation of Total Phenolic Content:
The total phenolic content of dry extracts was performed with Folin-Ciocaltaeu assay [13, 14, 15]. About 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23^0 C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample.

Estimation of Total Tannin Content:
The tannins were determined by Folin-Ciocalteu method [16]. About 0.1 gm of the sample extract was added to a volumetric flask containing 7.5 ml of distilled

water and 0.5 ml of Folin Ciocalteu phenol reagent, 1 mi of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Flavonoids determination by the method of Boham and Kocipai- Abyazan (1994) [17, 18]:
10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Statistical analysis: Analysis of results were statistically analyzed and given as mean (n=3) ±standard deviation of triplicate.

3. Results and Discussion

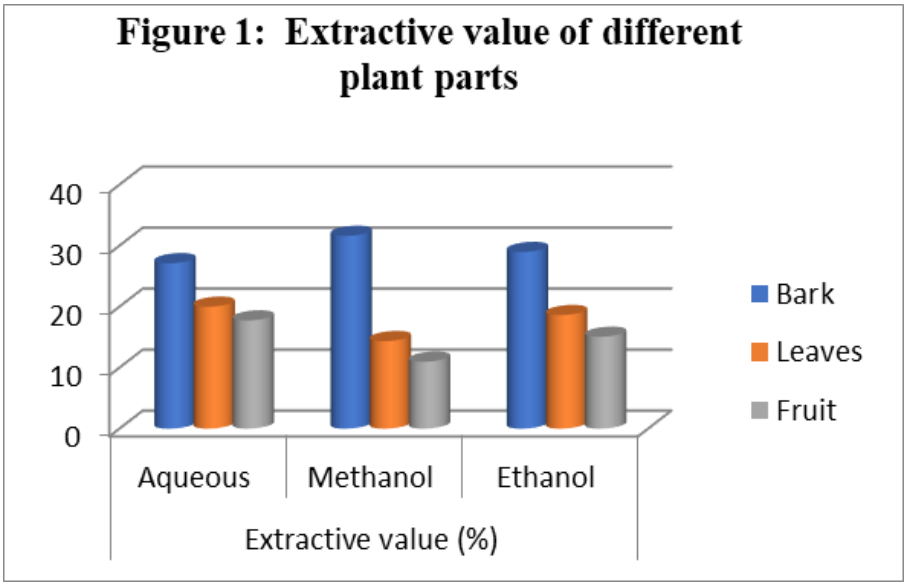
Qualitative phytochemical analysis:
The qualitative phytochemical analysis gives the array of various primary and secondary metabolite. Some concentration of proteins, carbohydrates, phenols, tannins, alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids, quinines, coumarins and pseudotannins etc. were recorded in varying level in different solvents used.

Table 1: Qualitative Phytochemical constituents of different plant parts of *T. arjuna*

Test	Bark			Leaves			Fruits		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
Tannins	+++	+++	+++	++	+++	++	+++	+++	+++
Saponins	++	++	++	++	++	++	+++	+++	+++
Flavonoids	++	++	++	++	++	+	+++	+++	+++
Terpenoids	+	+	++	+	+	++	++	++	+
Carotenoids	--	--	--	++	++	++	--	--	--
Cardiac Glycosides	+	++	+	+++	+++	+++	++	++	++
Quinones	++	+	+	--	--	--	+	+	+
Coumarins	++	++	+	++	+	+	+	+	++
Alkaloids	++	++	++	+	+	++	++	++	++
Pseudotannins	+	+	++	++	+	+	++	+	+
Phenols	+++	+++	+++	++	+++	+++	+++	+++	++
Resins	++	++	++	--	--	--	--	--	--
Fixed oils and fats	--	--	--	--	--	--	--	--	--
Acids	--	--	--	--	--	--	--	--	--
Proteins	+	+	+	++	++	++	++	+	+
Lipids	--	--	--	--	--	--	--	--	--
Cabohydrates	+	+	+	+	+	--	+	+	+

Table 2: Extractive values of different plant parts in different solvents

Plant part	Wt. of plant part(gms)	Extractive value (%)		
		Aqueous	Methanol	Ethanol
Bark	50	27.12	31.608	28.954
Leaves	50	19.988	14.352	18.62
Fruit	50	17.74	10.968	15.062



Extractive values:

The extract of leaves, bark, and fruits were prepared using three solvent i.e., distilled water, methanol and ethanol and the total extractive value calculated. The highest extractive value was found in ethanolic bark extract (28.954%) and lowest in methanolic leaf extract (14.352%) (Table 2, Figure 1). Extractive values gives the total possible biologically active percent of extract present in the sample [18], which get varied with the solvent.

Quantification of Total Phenolic Content, Tannin and Flavonoids:

The estimation of Total phenolic content, Tannin and Flavonoid content reveals that (Table III) higher concentration of total phenols found in bark and lower in fruits; Tannins found higher in leaves and minimum in fruits; higher amount of flavonoids in bark and lower in fruits (Figure 2, 3, 4).

Linear regression proves the reliability of results. Calibration plot of Gallic acid and Tannic acid gives R^2 value 0.9993 and 0.9992 respectively.

Table 3: Quantitative Phytochemical Estimation:

Plant Part	Total Phenols (mg/gm)	Tannins (mg/gm)	Flavonoids (mg/gm)
Bark	178.48±0.01	76.62±0.047	61.2±0.036
Leaves	144.64±0.015	83.67±0.03	51.3±0.025
Fruits	97.94±0.043	63.52±0.07	18.7±0.025

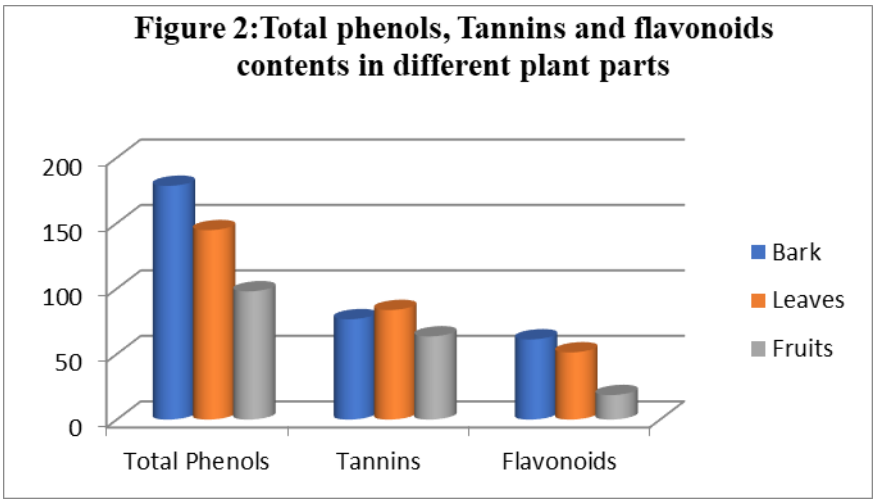


Figure 2:Total phenols, Tannins and flavonoids contents in different plant parts
200
150
100
50
0
Total Phenols
Tannins
Flavonoids
Bark
Leaves
Fruits

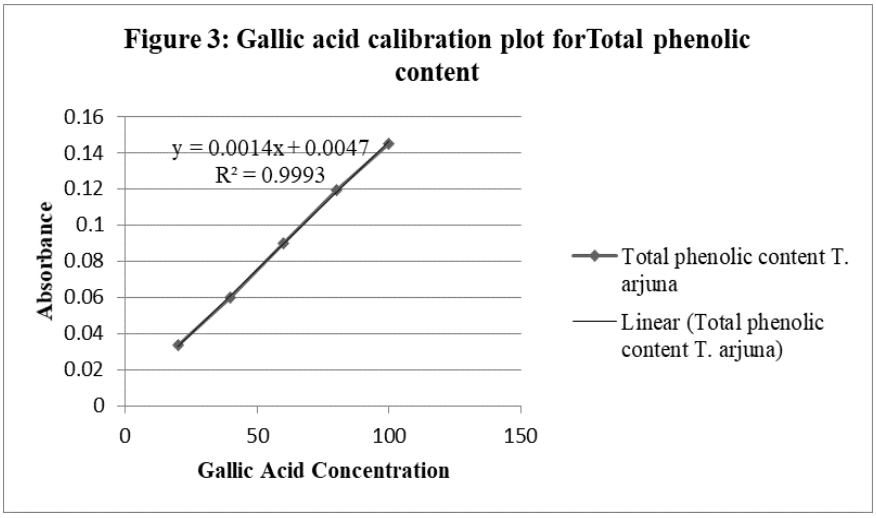


Figure 3: Gallic acid calibration plot forTotal phenolic content
y = 0.0014x + 0.0047
R² = 0.9993
Absorbance
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
50
100
150
Gallic Acid Concentration
Total phenolic content T. arjuna
Linear (Total phenolic content T. arjuna)

.

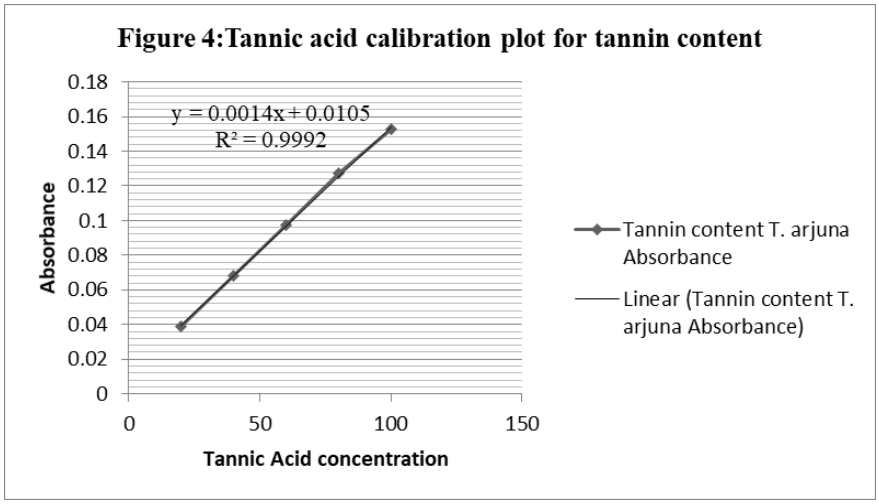


Figure 4:Tannic acid calibration plot for tannin content
y = 0.0014x + 0.0105
R² = 0.9992
Absorbance
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
50
100
150
Tannic Acid concentration
Tannin content T. arjuna Absorbance
Linear (Tannin content T. arjuna Absorbance)

The total phenols [19, 20], tannins and flavonoids [21, 22] from *Terminalia arjuna* have been studied by different workers at different geographical areas and found varying amounts of phytochemicals as it is dependent on different parameters like climate, soil nutrients, developmental stage, and processing methods of samples.

4. Conclusion

This study has shown that bark, leaves and fruits of *Terminalia arjuna* are the source of essential primary and secondary metabolites. The significant amount of total phenols, tannins and flavonoids contents hold up its traditional use in the treatment various diseases. Thus the phytochemical study of various parts of this plant will be helpful for further research formulation of therapeutics and drug development, as it been used in traditional healers medicine since ancient times by tribal people.

Conflict of interest

No conflict of interest influenced in this research.

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